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武庫川女子大紀要（自然科学）

Effect of Storage Time of the Solution for Gel Formation on SDS－PAGE* of Reduced Glutenin

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SDS－PAGE is usually used to determine the molecular weight of proteins and other analyses. In the present study we were aware that the old stock solution (mixture of all reagents for making gel except catalyst) gave significantly different electrophoregrams from those obtained with the fresh one. Only one week stock affected the result of SDS－PAGE.

Introduction

SDS－PAGE has frequently been used for analysis of proteins, such as check of impurities, determinations of number and molecular weight of components, and identification of subunit, etc. The authors have also used to analyze gluten proteins[1－6]. For preparing gel, we used the stock solution in which all reagents except catalyst were mixed as acidic PAGE[7]. As the result we obtained different electrophoregrams of reduced glutenin with time when the stock solutions were stored.

The present paper describes how the storage time of the stock solutions affected on electrophoregrams of reduced glutenin.

Materials and Methods

Gluten. Gluten was prepared from Columbus wheat flour (Canada Western Hard Spring Wheat) which was gifted by Canada Wheat Board, according to the modified method of Jones et al[8]. The wheat dough was hand－kneaded in 0.1% NaCl solution to remove starch and other impurities. The gluten ball was dispersed in 0.1N acetic acid solution to 15% (wet gluten, w/w) and centrifuged at 2,000×g and then the turbid supernatant obtained was centrifuged at 20,000×g for one hour. Clear solution obtained was heated over 70℃ for 10 min. After being heated, the solution was rapidly cooled and freeze－dried.

Glutenin. Glutenin (Gn) was prepared according to the method of Woychik et al[9]. The gluten obtained by the method described above was dissolved to 2% in 0.1N acetic acid and after ethanol was added to it up to 70% (v/v), pH of the solution was adjusted to 6.5 with 1N NaOH. After the solution was allowed to stand overnight in a refrigerator, it was centrifuged at 2,000×g for 10 min. The precipitate obtained was Gn, which was purified by repeating the same procedure.

Electrophoresis.

Preparation of separation gel. Stock solution (16.5ml) [acrylamide 16g, N, N'－methylenebisacrylamide 0.222g, SDS 0.133g, tris (hydroxymethyl) aminomethane 6.14g, TEMED 0.05ml : pH of the mixture was adjusted to 8.8 with 1N HCl and it was filled up to 100ml] was mixed with 5.5ml of 3% ammonium persulfate solution and

* SDS－PAGE : Sodium dodecyl sulfate－Polyacrylamide gel electrophoresis

poured into the gel−making plate. Final gel concentration was 12%.

Preparation of stacking gel. Stock solution (11.0 ml)[acrylamide 6.57g, N, N'−methylene-bisacrylamide 0.18g, SDS 0.15g, tris(hydroxymethyl)aminomethane 1.45g, TEMED 0.14ml:pH of the mixture was adjusted to 6.8 with 1N HCl and it was filled up to 100ml]was mixed with 5.5ml of 3% ammonium persulfate and poured onto the separation gel. Final gel concentration was 4.5%.

Sample for electrophoresis. Appropriate amount of sample was dissolved in 0.08M tris(hydroxymethyl)aminomethane − HCl buffer (pH6.8) containing 4% SDS, and then 2−mercaptoethanol was added to it up to 2%. After standing overnight acrylonitryl was added to it up to 4% because of preventing SH groups formed by reduction of disulfide groups from reoxidation.

Electrophoresis run. Electrophoresis run was made at the constant voltage, 33V/cm for stacking gel for one hour and 22V/cm for separation gel for 2 hours with a Slab Gel Electrophoresis Apparatus (Atto Co.). After running the proteins in the gel were fixed by trichloroacetic acid and stained by Coomassie Brilliant Blue G−250. All runs were carried out using the same sample and at nearly the same time.

Densitometry. The stained gels were subjected to densitometry with a Personal Densitometer (Molecular Dynamics Co.).

Results and Discussion

The results of SDS−PAGE of reduced Gn with the gels made of fresh and aged stock solutions are shown in Fig. 1. Relative mobilities of subunits detected by a densitometer were compared as shown in Fig. 2. The gel made of stock solution freshly prepared showed at least 15 subunits (bands) densitometrically, containing sample front. When the stock solution passed just one week, the number of subunit decreased to 12 and relative mobilities altered a little (B in Figs. 1 and 2). Older stock solution had stronger tendency above mentioned.

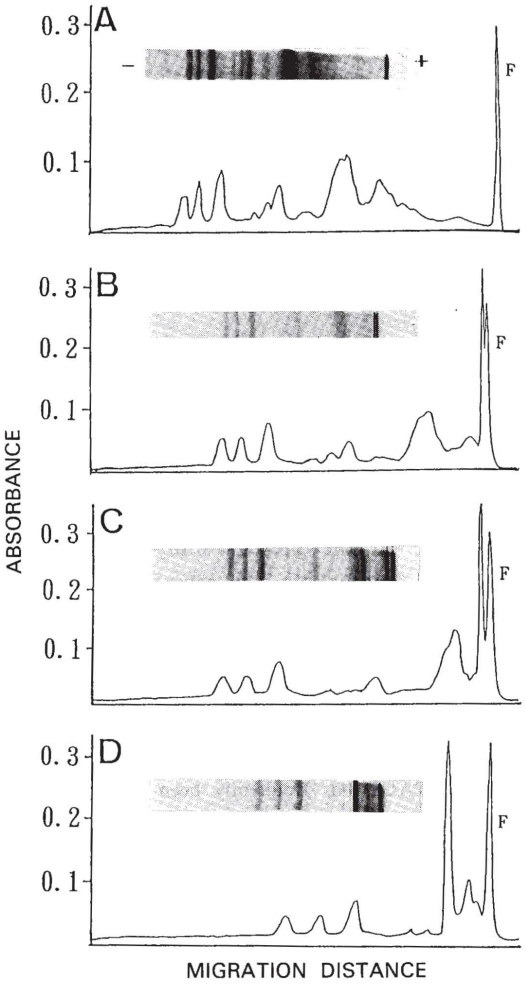

Fig. 1. Electrophoregram and densitogram in SDS−PAGE of reduced glutenin

A:gel prepared with fresh gel solution; B:with one week-passed gel solution; C:gel prepared with 4 week-passed gel solution; D: with 6 week-passed gel solution

Densitograms were obtained with a Personal Densitometer at 663nm of the wavelength. Electrophoresis runs were carried out at nearly the same time with the same sample

F shows the sample front

The gel formed with 4 week-passed stock solution showed 13 subunits and 16 week-passed one showed only 9 subunits and yet relative mobilities altered significantly. Not only subunit number but

migration distance was affected by storage period of the gel solution. Remarkable feature is that double line appeared at the sample front in SDS−PAGE using the gel solution to have passed only one week, and these lines were apart as seen in B to D in Fig. 1 and Fig. 2(shown with arrows).

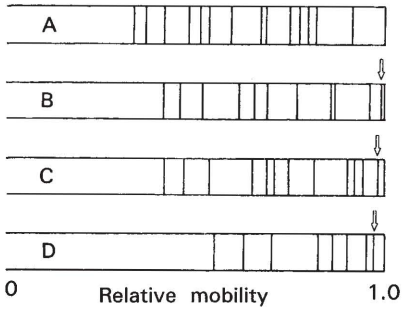

Fig. 2. Relative mobility of reduced glutenin in SDS−PAGE

Relative mobility of each subunit was calculated on the basis of the sample front in Fig. 1. The marks of A∼D have the same meanings as those in Fig.1. respectively.

In this experiment all reagents except ammonium persulfate of catalyst were mixed in the solution for gel formation. Since in acidic PAGE such procedure[7] is usually used, we tried to adopt it for SDS−PAGE. Although the reason why the phenomenon mentioned above occurs is not clear, obviously both methods have a risk obtaining wrong results in the electrophoregrams if the gel solutions are not freshly prepared. In the previous study, we did not use the recipi described in the present paper, and the results obtained in the previous experiment would be reliable.

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